Published March, 2014

RESPONSE OF FIVE CULTIVARS OF SUNFLOWER (*Helianthus annus*) TO ROOT-KNOT NEMATODE (*Meloidogyne spp*) INFECTION

Fabiyi, O. A.

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ABSTRACT

Dept of Crop Protection, University of Ilorin, Ilorin, Nigeria. Email: fabiyitoyinike@hotmail.com

Sunflower is well known for its allelopathic compounds which confers certain resistance to some pathogens on the plant. The susceptibility of sunflower cultivars to root-knot nematodes and response to control was investigated on the field. Five cultivars of Helianthus annus L, namely France lever, Isanka, Peredovicks, Pwk E5 and Sigco 445 were examined for their susceptibility to Meloidogyne spp, Alternaria leaf spot and virus mosaic. They exhibited different degrees of susceptibility to Alternaria leaf spot, Meloidogyne spp and virus mosaic. Most parameters of growth and yield that is; mean plant height, mean number of leaves, weights of head and seeds at harvest also differ significantly ($p<0.05$) among the cultivars. France lever appeared to be the least affected by the diseases and consequently exhibited superiority over others in most growth and yield parameters. The highest concentration of Eucalyptus officinalis powder reduced significantly ($p<0.05$) the pathogenic effect of Meloidogyne spp and other field pathogens on the plants which received them. The cultivars were examined quantitatively for the various phytochemical compounds present in them and France lever contains the highest degree of bio-active compounds, this explains the resistance ability of the cultivar, even under inoculated untreated conditions. Thus France lever is highly recommended for cultivation with the objective of vegetable oil production.

Keywords: Phytochemicals, France lever, Meloidogyne, Alternaria, virus mosaic, resistance.

INTRODUCTION

Sunflower, *Helianthus annus* L, an annual broad leaf herbaceous plant belongs to the family compositae. It is cultivated mainly for its edible oil seed (Kinman, 1963) and was introduced to Nigeria in the sixties to supplement groundnut oil and palm oil as a source of vegetable oil (Ogunremi, 1979). The production of vegetable oils is by far below current demand, since it covers about 10% of the consumption (El-Fayoumy *et al*., 1999). Sunflower is considered as one of the most important oil crops in the world today. Therefore, an improvement in the productivity of the crop is highly important, to meet the current shortage of vegetable oils for both home consumption and industrial use. Sunflower seeds have been estimated to have a feed constituent value of about 13.77% digestible crude protein, 14.05% fibre and 21.30% digestible oil (Oyenuga, 1968). It is also rich in polyunsaturated fatty acids, which is recommended as a vital part of the diet. Apart from the fatty acids, essential oil and nutritional components are obtained from sunflower oil. The plant also contain some bioactive compounds that have been associated with antibacterial activity, this include sesquiterpenes, lactones, annuionones, helibisabonols and heliannuols (Sankaranarayanan *et al*., 2008). Plant metabolites which include terpenoids, phenolics, carotenoids and allelochemicals have been identified and isolated from the plant leaves, stem and root. Other bio-active compounds stated to be present in sunflower include chlorogenic acid, caffeic acid, lutein, syringic acid, vanillic acid, ferulic acid, 2β, 16β-*ent*-kaurane diol, and 16α-epoxy-17β-al-*ent*-kaurane-19-oic acid (Canibe *et al*., 1999; Ghafar *et al*., 2001; Suo *et al*., 2006; Chethan and Gowda, 2010). Many of the compounds reportedly identified have been obtained in high concentrations especially in the root of the plant.

There are many cultivars of this plant which vary in height, size of head and oil content as well as time of maturity. However, all of them are propagated from seed which is normally sown early in the seasons for better performance (Ogunremi, 1979). The cake obtained after extraction is usually a rich source of protein and digestible fibre in livestock meal (Oyenuga, 1968). Like other plants sunflower is susceptible to many pests and diseases, some of which could lead to serious yield reductions. Among such pests are insects and birds, which destroy the seeds in the head especially when it is in the process of maturing. Plant pathogens associated with sunflower include fungi such as *Alternaria helianthi*, *Septoria* spp, *Verticillium* spp, *Sclerotia* spp and *Sclerospora* spp which cause diseases on leaves, shoot and root at various stages of growth. Caveness, (1967) reported the association of *Rotylenchus* spp, *Helicotylenchus* spp, *Xiphinema* spp and *Heterodera* spp among other nematodes of sunflower. Ogunremi, (1979) observed that low yield of sunflower in Southern Nigeria could be attributed to severe head rot under high humidity conditions prevalent during period of growth. A similar observation was made earlier in Canada by Hoes, (1969) who reported that severe head rot was prevalent in sunflower cultivated under prolonged wet weather and cool temperature conditions during summers. He also observed that sunflower cultivars grown on soils with high infestations of *Macrophomina phaseolina* had reduced seed yield and seed oil content. Other diseases of sunflower also include red rust caused by *Puccinia helianthi* which causes reduction in seed yield and seed oil content (Sackston, 1972). To combat the problem of diseases in

sunflower in cases where natural resistance may not be enough, the leaves of *Eucalyptus officinalis* was investigated as a possible form of bio-pesticide in view of the environmental pollution associated with the use of synthetic pesticides in general. *Eucalyptus officinalis* is a plant whose leaf essential oil have been known to have medicinal applications. It is used as a remedy for symptoms of respiratory infections such as colds, flu, and sinus congestion (Silva *et al*., 2003). The essential oils (EO) from the leaves have been established to have medicinal applications, including antibacterial and antifungal agents (Ramazani *et al*., 2002). Report demonstrating its insecticidal and nematicidal activity has also been stated (Brito *et al*., 2006). Khan *et al.*, 2008 found that the ethanolic extract of *Eucalyptus citriodora* applied at 1300kg/ha reduced the population of nematodes associated with papaya. In view of the importance of sunflower, and the prospects of its use, coupled with the fact that nematodes and other pathogens are factors militating against optimum yield in the plant. It is therefore necessary to investigate the pathogenic effect of *Meloidogyne* spp and other field pathogens on the plant, as they affect some growth and yield parameters and possible control methods as well as the resistant abilities of the various cultivars and investigate the quantity of the plant metabolites which has conferred the resistance on them.

MATERIALS AND METHODS

Field experiment

A field experiment was carried out at the University of Ilorin Teaching and Research farm, Ilorin (Lat 8° 29' N of the Equator; Long: 4°, 40' E of the Greenwich Meridian), in the southern guinea savannah ecological zone. The experiment was a randomised block design of five treatments, each replicated four times, thus making a total of twenty (20) plots measuring 25 m^2 each. The populations of *Meloidogyne* spp in the soil was augmented by inoculating with galled roots of okra. Planting was done immediately by sowing three (3) seeds per hole at a spacing of 25 cm within and 75 cm between rows. These were later thinned down to a plant per stand three weeks after planting (WAP). *Eucalyptus officinalis* powder was weighed out at 75g, 55g, 35g and 25g. This was applied to another set of 20 plots of sunflower each replicated four times, while the 10 plots of inoculated untreated plots and un-inoculated not treated plots served as control. Observation was made on the resistance ability of each of the sunflower cultivars, and the response of the sunflower cultivars under series of infection to control using *Eucalyptus officinalis* thus a total of fifty (50) plots were used in the experiment. Soil samples were also taken from the un-inoculated plots to access the native nematode population on the field. The plant materials were applied in banded form at the base of each plant on the plot. Plant height, number of leaves per plant, number of leaves with fungal leaf spots and number of leaves with virus mosaic disease were recorded over a six week period starting from the 7th week after planting on all the experimental section. Fifty (50) plant stands which were tagged randomly per plot were used for data taken from the 50 plots of the experiment. Harvesting was done at the 15th week after planting, by uprooting each tagged plant wholly; head weight, seed weight, and root weight were then taken.

Laboratory experiment

The initial and final nematode populations in the soil were estimated by extracting with the modified Baermann's technique (Whitehead and Hemming, 1965). Nematode populations were then counted under the stereomicroscope. Roots were rated for galls using the 0.5% scale after Taylor and Sasser (1979).

Quantitative analysis of phytochemicals

The roots of *Helianthus annus* were analysed for the quantity of phytochemicals contained in them.

Flavonoids: Twenty grams (20 g) each of the chopped pieces of roots were weighed separately into 2M hydrochloric acid; this was heated on the water bath for 35minutes. The extract was filtered with Whatman's no 1 filter paper and weighed.

Alkaloids: Twenty gram (20 g) of the plant material was weighed into a conical flask and 200 mL of 10% acetic acid in ethanol was added and allowed to stand for four hours. This was filtered and concentrated over water bath. Five drops of ammonium hydroxide was added to achieve precipitation while stirring on the magnetic stirrer. The precipitate was later filtered and this was taken as the crude alkaloid (Harborne, 1973).

Phenols: The plant materials (20 grams each) were boiled with ether in a conical flask. 15 mL of the extract was taken into a 100 mL flask and 5 mL of distilled water was added. 5 mL of alcohol and 2 mL of ammonium hydroxide was also added and the content of the flask was left for a while for a colour reaction to take place (Obadoni and Ochuko, 2001).

Saponins: Each plant material was soaked in 100mL ethanol and stirred on the magnetic stirrer for 6 hrs at 55°C. The mixture was filtered and the residue was extracted again with aqueous ethanol. The ethanol extract was concentrated on water bath. The extract was partitioned into diethyl ether in a separating funnel. The ether layer was discarded while the aqueous layer was recovered and 60 mL of n-butanol was added and later washed with 5% sodium chloride. The sample mixture was dried in the oven to a constant weight and the percentage saponin was calculated (Obadoni and Ochuko, 2001).

Data analysis
All data were subjected to analysis of variance and significant means were separated using the new Duncan's multiple range test at 5% level of probability.

RESULTS

The phytochemicals from the roots of *H. annus* were a powdery substance appearing yellowish and colourless respectively, and they were completely soluble in water.

Table 1: Phytochemicals in the roots of different cultivars of *Helianthus annus*

Varieties	Alkaloids (mg)	Flavonoids (mg)	Saponins (mg)	Tannins (mg)
France lever	82	91	87	81
Peredovicks	73	80	80	74
Sigco445	61	72	76	68
PwkE5	54	63	70	63
Isanka	49	52	64	57

From Table 1 France lever contains the highest quantity of phytochemicals among all the other varieties tested, while Isanka had the least. Analysis of nematode populations in 250g soil before planting revealed that some of the plots were significantly ($p<0.05$) more infested than the other before planting. Plots grown to France lever had the highest number of nematodes with a mean population of 2282, followed by that of Peredovicks-2215; Isanka-1923; Pwk E5 1243 and Sigco 445-1105 (Table 2). This indicates that the plants in each plot had the same chance of being infested all things being equal. The population of nematodes in the soil reduced at harvest compared to what it was before planting, thus signalling the effect of the control material used. Plots grown to France lever had the highest apparent mean populations in the soil even at harvest with a mean of 319 nematodes, while PwkE5 had the least, there was however significant ($p<0.05$) differences in nematode populations between some of the plots. All the varieties were susceptible to root knot nematode *Meloidogyne* spp since all plants were galled either moderately or heavily. France lever had the lowest mean gall index of about 0.15 while Isanka had the highest. Nematode population analysis of un-inoculated plots revealed the absence of *Meloidogyne incognita* but *Scutellonema bradys* was identified. The effect of the different level of application of treatment material was significant ($p<0.05$) on the yield of the cultivars. Plants which received the highest level of application (75g) had significantly higher vegetative growth. The mean plant height of the different varieties are shown in Table 3, all plants increased in height steadily to the 12th week after planting (WAP), but France lever was significantly taller than the other four varieties. Table 4 shows the mean number of leaves among the varieties; France lever had the highest number of leaves throughout the period of observation, while PwkE5 and Isanka had the least. The mean number of leaves that were infected with *Alternaria* leaf spot is significantly fewer in peredovicks and France lever, while Isanka had the highest. Table 6 depicts the mean number of leaves infected with mosaic and yellowing. Isanka and PwkE5 were observed to have significantly higher number of leaves with virus induced mosaic, while France lever had significantly lower number of leaves. France lever also had significantly ($p<0.05$) heavier head, seed, stem and root weight, compared to the other cultivars. The rate of treatment application was also significant on the incidence of *Alternaria* leaf spot, mosaic attack and root galling, each disease control was dose dependent irrespective of cultivar. Plants treated with the highest concentration had significantly ($p<0.05$) fewer incidences of *Alternaria* leaf spot, virus mosaic and root galling (Table 7). Sunflower plants in the inoculated untreated plots (control plots) were seriously damaged by *M. incognita* and other pathogens, but France lever displayed cultivar resistance, which was followed by peredovicks. The plants in the un-inoculated and untreated plots had minimal incidence of virus mosaic and *Alternaria* leaf spot attack on the field.

Table 2: Nematode population before planting, at harvest and root gall index

Varieties	Nematode population in 250g soil before planting	Nematode population in 250g soil at harvest	Root gall index
France lever	2282^{a}	319^{c}	0.15^{a}
Isanka	1923^{b}	310^{c}	3.21^{bc}
Peredovicks	2215^{a}	205^{b}	2.05^{b}
PwkE5	1243^{c}	111^{a}	2.24^{b}
Sigco445	1105^{d}	211^{b}	2.12^{b}

Means in a segment of a given column followed by the same letter are not significantly different at $p<0.05$

Table 3: Plant height of sunflower under nematode infection

Varieties	7th wap	8th wap	9th wap	10th wap	11th wap	12th wap
France lever	102.12[a]	116.19[a]	129.36[a]	138.21[a]	151.07[a]	160.22[a]
Peredovicks	90.03[b]	99.63[b]	106.29 [b]	116.19[b]	130.09[b]	142.18[b]
Sigco445	b84.25[c]	91.26[c]	98.67[c]	107.18[c]	123.04[c]	136.40[c]
PwkE5	79.00[d]	88.22[d]	97.39[d]	103.01[d]	115.52[d]	125.10[d]
Isanka	71.87[e]	80.43e	91.22[e]	99.04[e]	108.27[e]	118.28[e]

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05

Table 4: Number of leaves of sunflower under nematode infection

Varieties	7th wap	8th wap	9th wap	10th wap	11th wap	12th wap
France lever	22.03[a]	27.00[a]	32.17 [a]	36.19[a]	37.10[a]	40.22[a]
Peredovicks	16.21[b]	19.17[b]	23.06[b]	30.15[b]	33.18 [b]	36.09[b]
Sigco445	15.11[b]	18.20[b]	20.22[c]	26.06[c]	30.00[c]	33.00[c]
PwkE5	13.03[c]	15.14[c]	19.10[c]	21.11[d]	25.02[d]	28.15[d]
Isanka	10.22[d]	12.09[d]	16.31[d]	18.34[e]	23.11[e]	25.00[e]

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05

Table 5: Incidence of *Alternaria* leaf spot disease on sunflower under nematode infection

Varieties	7th wap	8th wap	9th wap	10th wap	11th wap	12th wap
France lever	0.13[a]	0.26[a]	0.38[a]	0.51[a]	0.42[a]	0.62[a]
Peredovicks	2.01[b]	3.11[b]	3.89[b]	2.26[b]	2.12[b]	1.22[b]
Sigco445	2.32[b]	4.07[b]	5.20[c]	4.05[c]	4.00[c]	5.11[c]
PwkE5	4.21[c]	6.29[c]	8.06[d]	6.12[d]	6.20[d]	7.21[d]
Isanka	6.22[d]	8.09[d]	11.12[e]	8.15[e]	9.02[e]	9.00[e]

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05

Table 6: Incidence of leaf mosaic and yellowing on sunflower under nematode infection

Varieties	7th wap	8th wap	9th wap	10th wap	11th wap	12th wap
France lever	0.08[a]	0.15[a]	0.39[a]	0.48[a]	0.41[a]	0.25[a]
Peredovicks	0.11[b]	0.20[a]	1.23[b]	1.78[b]	1.26[b]	1.13[b]
Sigco445	1.12[c]	1.34[b]	2.01[c]	2.59[bc]	2.29[c]	2.20[c]
PwkE5	1.07[d]	1.30[b]	2.14[c]	3.18[c]	3.27[d]	3.30[d]
Isanka	2.24[e]	2.54[c]	3.13[d]	4.29[d]	4.00[e]	4.57[e]

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05

Table 7: Head, seed, stem and root weights of sunflower under nematode infection

Varieties	Head	Seed	Stem	Root
France lever	49.32a	36.14a	43.22	27.10a
Peredovicks	31.23b	29.04b	34.05	21.43b
Sigco445	24.18c	22.07c	28.32	15.04
PwkE5	19.28d	18.10d	21.28	11.38
Isanka	16.00e	14.19e	17.12	6.12e

Means in a segment of a given column followed by the same letter are not significantly different at p<0.05

DISCUSSION

The varieties of sunflower investigated exhibited different responses to *Meloidogyne* spp and other pathogenic attack during their period of active growth. France lever appeared to be highly resistant to attack by producing significantly higher plant height, number of leaves, fewer numbers of leaves attacked by *Alternaria* and virus mosaic, and also showing superiority over others in yield parameters such as the head, seed, stem and root weights which were significantly heavier than the other cultivars. Among the plants in the inoculated untreated plots and un-inoculated untreated plots, France lever exhibited cultivar superiority by displaying resistance to *M. incognita* and infection from other field pathogens, head and seed weight was superior among all the cultivars under this condition. Studies have indicated that only a few variety of sunflower are resistant to root-knot nematode *Meloidogyne* spp. Vinconte (1982) had observed that most sunflower varieties are not highly resistant to *Meloidogyne incognita*, while Rich and Dunn (1982) reported that though many nematodes reproduce well on

the crop, the crop appeared to be tolerant to parasitism by some nematodes including *M. incognita*. In this study, it was observed that the plants were susceptible to attack in varying degrees but some seems more tolerant than the other. This may be attributed to the presence of some bio-active compounds in various amounts in the cultivars as revealed by the result of the quantitative phytochemical screening. The resistance exhibited by France lever is not unconnected with the degree of phytochemicals in the root, as depicted in the quantity of phytochemicals present in this particular cultivar. These phytochemicals have been associated with nematicidal activity. The levels of naturally occurring phenols in roots have been correlated with resistance in certain plant cultivars to nematodes (Narayana and Reddy, 1980). Odeyemi and Adewale (2011) also established that reproduction and galling were significantly suppressed when ethanol and aqueous extract of *Tithonia diversifolia* was applied on *M. incognita* infecting yam in the screen-house. They reported the constituents of the ethanol extract to be saponin and alkaloids. France lever is recommended for commercial vegetable oil production because of the promising high yield and ability to resist infection with or without control. However the leaves of *Eucalyptus officinalis* is also recommended for *Meloidogyne incognita* control in infected agricultural fields.

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